NF-κB signaling and human disease

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Despite substantial progress in understanding the NF-κB signaling pathway, the connections between this pathway and human disease are only now being elucidated. Genes that function within or upstream of the NF-κB pathway have been found to cause four distinct disorders and two allelic conditions. Investigation of these genes and disorders has brought significant insight into the role of NF-κB in various aspects of physiological development.

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Abbreviations

ADED	autosomal dominant hypohidrotic ED
ARED	autosomal recessive hypohidrotic ED
ED	ectodermal dysplasia
EDA	ectodysplasin A
EDAR	ectodysplasin A receptor
FEO	familial expansile osteolysis
IKK	IκB kinase
IP	incontinentia pigmenti
NEMO	NF-κB essential modulator
NF-κB	nuclear factor κB
PDB2	Paget disease of bone 2
PL	primary lymphedema
RANK	receptor activator of NF-κB
TNF	tumor necrosis factor
TNFR	TNF receptor
VEGFR	vascular endothelial growth factor receptor

Introduction

Since the discovery of nuclear factor κB (NF-κB) in 1986 by Sen and Baltimore [1], tremendous effort has been expended to elucidate the functions of this transcription factor. NF-κB consists of homodimers or heterodimers of a family of proteins that share a common Rel homology domain (RHD) that consists of DNA-binding and dimerization domains. NF-κB is primarily involved in inducing immune and inflammatory responses [2–4] and in regulating apoptosis [5,6]. Its targets include genes that produce cell adhesion molecules, cytokines, chemokines, and anti-apoptotic factors. The inhibitory IκB molecules sequester NF-κB in the cytoplasm by masking its RHD. When a cell is stimulated by cytokines, such as interleukin-1 or tumor necrosis factor α (TNF-α), a second complex called IκB kinase (IKK) is activated. The γ subunit of IKK (NF-κB essential modulator [NEMO]) is the regulatory component, and the α and β subunits have kinase functions. The activated IKK complex phosphorylates IκB, preparing it for degradation by ubiquitin-mediated proteolysis. NF-κB then translocates into the nucleus to activate transcription of target genes (Figure 1) [7••,8••].

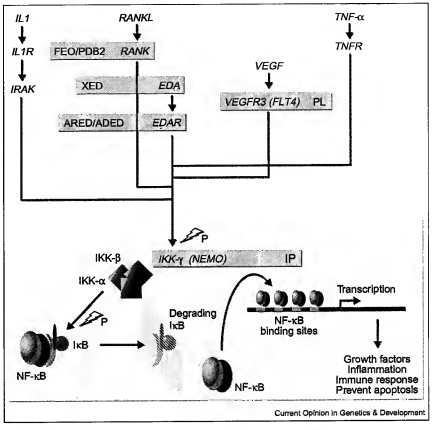
Despite its involvement in fundamental cellular processes, NF-κB signaling has rarely been studied in depth in the human context due to a lack of connections to distinct human diseases. However, in the past year several publications have described defective NF-κB function in genetic disorders, including ectodermal dysplasia (ED), familial expansile osteolysis (FEO), primary lymphedema (PL) and incontinentia pigmenti (IP). In this review, we will describe the genetic mutations that cause these four disorders, and the specific alterations in the NF-κB pathway. Relevant mouse models will also be described, as defects in NF-κB function affect various physiological systems and organs in mice, including the immune system [9,10], fetal liver [11], skin [12], limbs [13], and the osteoclast lineage [10]. While the knowledge of the NF-κB pathway has helped explain the pathogenesis of ED, FEO, PL and IP, the human disease phenotypes have also provided significant insight into the pathway itself.

Ectodermal dysplasia

Over 150 distinct phenotypes are classified as ectodermal dysplasias based on a presentation of abnormal teeth, skin, nails, sweat glands and hair. Patients typically exhibit sparse hair, decreased sweating and irregular pigmentation. Only three forms of ED are known to result from defects in NF-κB-associated functions (Table 1), including X-linked anhidrotic ED (XED; Christ–Siemens–Touraine syndrome; OMIM 305100), autosomal recessive hypohidrotic ED (ARED; OMIM 224900), and autosomal dominant hypohidrotic ED (ADED; Clouston syndrome; OMIM 129490 and 129500). The XED phenotype resembles that of the *Tabby* mouse, and both are due to mutations in a protein called ectodysplasin A (EDA), which has significant similarity to TNF [14,15]. Alternative splicing produces two differentially expressed isoforms of EDA — EDA-A1 and -A2. They differ by two amino acids that specify receptor binding, such that EDA-A1 binds the EDA receptor (EDAR) whereas EDA-A2 interacts with the product of the X-linked ectodysplasin receptor gene (XEDAR; OMIM 300276), the product of a separate X-linked gene [16]. Mutations in *XED* and *Tabby* are scattered throughout the respective genes with most found in collagenous repeats, whose functions remain to be understood.

ARED and ADED have corresponding mouse phenotypes in the form of *downless* (*dl*) and *Sleek* (Dl^{Slk}), respectively (Table 1). Human pedigrees segregating ADED typically show better heat tolerance and less hair loss than families with ARED, and XED is clinically indistinguishable from ARED. Interestingly, ARED and ADED are allelic, both in humans and mice, since they result from mutations in the same EDAR. This receptor contains a death domain (PS50017) that is characteristic of the TNF receptor (TNFR) family [17••] and is expressed during embryonic growth in

Figure 1



Upstream activators of NF-κB signaling. Human diseases and their corresponding genes are shown in grey boxes. These boxes are positioned at locations within pathways where the genes function. Thus, NEMO acts as the central regulator of the NF-κB pathway and the other genes mediate their effects via NEMO. Note that the receptors affected in ARED/ADED, PL, and FEO/PDB2 are all members of a family that includes TNFR, which also acts via NF-κB. Since multiple pathways impinge on the NF-κB transcription system, it is conceivable that combinations of mutations in the upstream genes could cause a phenotype similar to that produced by specific defects in *NEMO*. IL1, interleukin-1; IL1R, IL-1 receptor; P, phosphorylation; RANKL, osteoprotegerin ligand.

cell lineages that specify the formation of teeth, sweat glands and hair [18••]. The dominant mutations in EDAR are predicted to either remove or damage the death domain and are thus likely to hinder normal function in multimerization and downstream signaling [19]. Similarly, the dominant *downless* mouse phenotype, *Sleek* (Dl^{slk}), arises from disruption of the cytoplasmic portion of EDAR, which contains the death domain at its carboxyl terminus end [18••]. In general, recessive mutations are located in the TNFR-like extracellular domain and are likely to affect interactions with upstream signaling molecules. *In situ* hybridization analysis has shown homogeneous expression of *Edar* in mutant mouse embryos and restricted expression in maturing follicles in wild-type embryos, indicating that EDAR could signal the development of hair follicles during embryonic growth [18••]. EDAR also activates NF-κB, as well as other transcriptional regulation pathways [20], although the precise downstream consequences remain to be determined. Therefore, it is likely that mutations in *EDA* or *EDAR* prevent activation of target genes by NF-κB and thus affect the embryonic development of skin, hair and sweat glands.

Familial expansile osteolysis and familial Paget disease of bone

Bone construction depends on a fine balance between ossification and resorption, two processes controlled by distinct signaling pathways. Mutations in the TNFR-like molecule RANK (receptor activator of NF-κB), encoded by the *TNFRS11A* gene, cause autosomal dominant FEO (OMIM 174810) and Paget disease of bone 2 (PDB2; OMIM 602080). This disorder is characterized by progressive resorption of osteoclasts, medullary bone expansion that leads to painful and disabling deformities, and a susceptibility to pathologic fracture [21••] (Table 1). FEO and PDB2 are distinguished by the location of osteolytic lesions, with damage to the appendicular skeleton in FEO, and to the axial skeleton in PDB2. However, both FEO and PDB2 families exhibit deafness and abnormal dentition. Although different mutations have been identified in FEO patients than in PDB2 patients, all mutations prevent cleavage of the RANK signal peptide, and this is predicted to prevent the mutant proteins from reaching the cell surface. RANK is essential in mediating the effects of the osteoprotegerin ligand (OPGL/RANKL) in the generation of osteoclasts, which are required for resorption during bone remodeling [22,23•]. The intracellular accumulation of defective RANK proteins in patients' cells leads to an increase in NF-κB activity, suggesting that the FEO/PDB2 mutations create dominant activating proteins [21••]. The connection between RANK and NF-κB is further supported by evidence that a potentially destabilizing mutation in NEMO, a central regulator of NF-κB, causes osteopetrosis [24••],

Table 1

Genes in the NF-κB pathway and related human diseases and mouse models.

Gene	Location*	Human disease	Mouse model	References
Core genes in the NF-κB pathway				
IKK-α (IKK1/CHUK)	10q24–q25	No association; lethal restrictive dermatopathy?	KO: epidermal hyperproliferation	[52,54]
IKK-β (IKK2)	8p11.2	No association	KO: lethal-liver apoptosis	[50,51]
IKK-γ (NEMO)	Xq28	IP; ED-ID; OL-ED-ID;	KO: male lethal-liver apoptosis, heterozygous female IP	[24,42–45]
P50/p105 (NF-κB1)	4q24	No association	TG: defective immune response	[49] (review)
P52/p100 (NF-κB2)	10q24	No association; leukemia or non-Hodgkin lymphoma?†	KO: lethal-liver apoptosis	[49] (review)
Rel-A/p65 (NF-κB3)	11q13	No association; Crohn's disease?‡	KO: lethal-liver apoptosis	[11,60]
Rel-B (IREL)	19	No association	None	
IκB-α (NF-κBIA)	14q13	No association	KO: dermatitis	[49] (review)
IκB-β (NF-κBIB)	19q13.1	No association	None	[49] (review)
Genes upstream of the NF-κB pathway				
ED1	Xq12–q13.1	X-linked hypohidrotic ectodermal dysplasia	*Tabby*: lack normal hair, sweat glands, teeth	[14,15]
EDAR (DL)	2q11–q13	Autosomal recessive and dominant anhidrotic ED	*Downless*: lack normal hair, sweat glands, teeth	[17,18]
XEDAR	X	None	None	
TNFRSF11A (RANK)	18q21.2–q21.3	Familial expansile osteolysis or Paget disease of bone 2	KO: abnormal osteoclast function	[21–23]
TRANCE (RANKL/OPGL)	13q14	No association; osteopetrosis and chondrodysplasia?	KO: osteopetrosis, chondrodysplasia, anodontia	[25,61]
VEGFR-3 (FLT-4)	5q35.3	Primary lymphedema	KO: lethal, abnormal angiogenesis	[29]
IRAK	Xq28	No association; incontinentia pigmenti?	KO: immune dysfunction	[62,63]

There are numerous upstream activators and repressors of NF-κB but only some are listed because of mouse phenotypes that could be relevant to human disease. No association: by linkage or mutations. KO, knockout mice; TG, transgenic mice; *human cytogenetic location; †inferred from translocation cases; ‡from mouse model of experimental colitis. ? indicates speculative association.

similar to mice lacking the *Rank* gene [25]. In addition, the development of osteoclasts has been shown to depend on NF-κB-induced transcription of target genes [10,26]. Other factors besides NF-κB, such as Jun N-terminal kinase (JNK) [23•], are likely to implement the effects of RANK-dependent signal transduction on bone metabolism. Therefore, although alterations in NF-κB activity might offer initial clues to explain the osteolysis in FEO/PDB2 or the osteopetrosis resulting from *NEMO* mutations, it is likely that these phenotypes arise from a more complex disturbance of RANK-mediated pathways.

Primary lymphedema

During development, angiogenesis depends on interactions between several vascular endothelial growth factors (VEGFs) that implement their effects through multiple receptor tyrosine kinases, including VEGF receptor 1 (VEGFR1), VEGFR2 and VEGFR3 [27,28]. VEGF-C and VEGF-D bind VEGFR3 and regulate vascular development in the embryo and in adult tissues. Mutations of *VEGFR3* in humans cause autosomal dominant PL (OMIM 153100), which is characterized by dilation of lymph capillaries due to internal capillary edema and enlargement of interendothelial spaces [29••] (Table 1). In addition, the *Vegfr3*-null mouse dies at embryonic day 9.5 from incomplete vascular development [30]. VEGFR3 is a receptor tyrosine kinase required for the differentiation of endothelial cells in blood and lymphatic vessels. Binding of ligand activates the intracellular catalytic domain of the receptor, which induces downstream signaling pathways, including that of NF-κB [29••]. The substitution mutations identified in PL patients are dominant-negative types that produce receptors without the capacity for tyrosyl autophosphorylation but with increased resistance to degradation. Although the mutant receptors are more stable, and therefore supplant the wild-type receptors, they have diminished ability to induce NF-κB signaling [29••] and thus cause PL. Similar mutations in *KIT*, a close relative of *VEGFR3*, cause human piebaldism, which is characterized by hypopigmentation of the skin and hair, and by abnormal development of germ-cell, hematopoeitic, and melanocyte lineages [31]. However, it is not yet known whether *KIT* implements its effects via the NF-κB pathway.

Incontinentia pigmenti

The disorder that provides the best insight into the NF-κB pathway is IP (OMIM 308310), an X-linked dominant and typically male-lethal disorder [32] (Table 1). Affected females survive because of dizygosity for the X chromosome and selective proliferation of cells expressing the normal X chromosome; therefore, IP females demonstrate complete skewing of X-inactivation in favor of the mutant X chromosome [33–35], a feature often used to confirm diagnosis. Newborn female patients exhibit a characteristic

four-stage skin pigmentation abnormality that begins with erythematous skin lesions and ends with hypopigmented, marble-cake-like swirls. The most significant problems associated with IP are blindness, due to retinal detachment, and central nervous system disturbances [36–39]. Minor signs include hair loss, conical teeth and nail dystrophy. This multisystemic nature leads to difficulty in diagnosis, which is compounded by significant variation in expressivity, sometimes within the same family.

The male lethality and skewing of X-inactivation in females suggests that the IP gene is vital for cell survival and fetal development. It was shown recently that IP results from enhanced apoptosis due to mutations in the *NEMO* (*IKK-γ*) gene [24••]. Approximately 85% of patients carry an identical deletion that removes most of the gene. Extensive biochemical analyses of NEMO have shown that it is indispensable for NF-κB activation [40,41] (Figure 1). Cells from typical IP patients lack NF-κB activity, due to the absence of NEMO, and are susceptible to TNF-α-induced apoptosis, although other components of the NF-κB pathway are unaffected [24••]. In support of the human mutation data, two groups subsequently described the phenotype of *Nemo* knockout mice [42••,43••], and as expected, males do not survive without *Nemo* function and die from liver apoptosis at embryonic day 13, similar to NF-κB (*p65*) and *IKK-β* knockout mice (Table 1). The heterozygous females have a phenotype that is highly similar to human IP, including skin lesions, incontinence of melanin, granulocyte infiltration, and the presence of detached keratinocytes in the epidermis. A third group also described the male lethality in *Nemo*-null mice, but an IP phenotype was not found in females, possibly due to a different mouse strain background [44]. The retinal and central nervous system problems found in humans have not been observed in $Nemo^{+/-}$ mice yet. The pathogenesis of IP will be better understood when we elucidate the time and location of *NEMO* expression and identify the target genes downstream of NF-κB.

Variants of Incontinentia pigmenti and ectodermal dysplasia

Loss-of-function mutations in *NEMO* are uniformly lethal in males but we recently identified three male patients with nonlethal mutations. These males had survived to term and demonstrated IP signs along with disrupted hemostasis alone, or in combination with immune dysfunction, colonic bleeding and osteopetrosis [24••,45••]. Affected female relatives demonstrated classic IP but none of the atypical signs seen in the males. Analysis of *NEMO* revealed mutations within the last exon, which encodes part of the carboxyl terminus of NEMO, known to be indispensable for NF-κB activation [40,41]. These male IP mutations were considered hypomorphic because they did not cause skewing of X-inactivation in females and did not eliminate NF-κB activity, thereby explaining why the males survived. Such male patients provide valuable insight into the roles of NF-κB since the complete effects of their mutations are exposed. In contrast, typical IP mutations are cell-lethal, and thus, full expression is prevented because it would be lethal in males and cause skewed X inactivation in females.

Since IP was traditionally thought to be male-lethal, and because surviving males exhibit additional signs not commonly associated with IP, male cases are likely to be misdiagnosed with a disorder similar to IP, such as ED. Only two of our male patients suffered from immune dysfunction, and one of them was initially diagnosed with ED [45••]. Because *NEMO* mutations were discovered in both males, it was predicted that immune dysfunction associated with apparent ED is simply an atypical presentation of IP. Indeed, recent analyses of male patients with immune dysfunction associated with ED failed to identify mutations in *ED1*, the gene for X-linked ED; but instead, these males showed mutations in *NEMO* [46••]. In addition, two other publications have recently described mutations of *NEMO* in male patients with ED and immune deficiency (ED-ID) [47••,48••], or a combination of ED-ID, osteopetrosis, and lymphedema (OL-ED-ID) [47••], similar to a previous report [24••]. Interestingly, all of the mutations described in these atypical IP/ED male patients to date have been in the carboxyl terminus of NEMO, and they do not completely abolish NF-κB activity. Further investigation of these mutations will significantly improve our understanding of the roles of NF-κB in the various physiological systems affected in these males.

Although some of these conditions have been described as novel syndromes, it is important to understand that in some cases, where affected female relatives of the male patients were present, the same mutations caused a typical IP phenotype in the female relatives. Thus, hypomorphic mutations of *NEMO* lead to variant signs in males as a result of the full phenotypic expression of the mutations in the absence of a normal X chromosome, and cause a classic IP phenotype in female individuals. In short, males with *NEMO* mutations can either have IP and hemorrhaging [24••,45••], IP or ED combined with immune dysfunction, osteopetrosis, and lymphedema [24••,47••], or ED with immune dysfunction only [45••,46••,48••]. In order to avoid confusion during diagnosis, it might be prudent to use a comprehensive term to describe these overlapping combinations of IP, ED, ED-ID, and OL-ED-ID. We propose 'VOIMIE syndrome' (for 'vascular anomalies, osteopetrosis, or immune dysfunction in males with IP or ED').

Mouse models for genes in the NF-κB pathway

Mouse models have provided valuable insights into the NF-κB pathway. Mice lacking specific subunits of NF-κB die during embryonic development from massive liver apoptosis (see Table 1 and [49] for review). Elimination of *IKK-β* also causes the same phenotype [50,51], further emphasizing that this protein is necessary for NF-κB activation. In contrast, $IKK\text{-}\alpha^{-/-}$ mice survive to term and demonstrate a hyperproliferative and sticky epidermis, and

lack normally extended limbs [52–54]. Histological analysis has shown that the basal keratinocytes in these mice undergo uninhibited proliferation. This phenotype is reminiscent of a human condition called 'lethal restrictive dermatopathy' (LRD; OMIM 275210). Thus, examination of LRD patients for mutations in the human *IKK-α* gene, located in 10q24–q25, is warranted. The phenotypes of transgenic mice with altered NF-κB expression in the epidermis further support the significant involvement of NF-κB in skin development [12,55•,56,57]. Specifically, reduced expression of NF-κB causes hyperproliferation of the epidermis because of the lack of cell-cycle arrest of keratinocytes, prior to terminal differentiation in keratinized outer cell layers. In contrast, an increase in expression causes hypoproliferation of the epidermis [53].

The various disorders described in this review provide substantial insight into the functions of NF-κB. Because NEMO is a central regulator of NF-κB, IP provides a central hub from which to study other diseases with phenotypic similarities. Disorders in which the genes implement their effects via NEMO could be characterized by defects sometimes seen in IP. For instance, IP and ED both exhibit skin pigmentation abnormalities that likely arise from similar defects in NF-κB signaling. Second, the osteoclast defects in one rare IP male [24••] resemble those of FEO/PDB2 patients, so the pathogenesis could be related in these cases. Lastly, the hemorrhaging seen in two IP males [24••,45••] could reflect problems with VEGFR3 signaling, similar to what is observed in PL patients. The immune dysfunction observed in other males, however, appears restricted to male IP and it is likely to involve a pathway that excludes EDA, RANK and VEGFR3. Therefore, it is conceivable that a combination of EDA, FEO, and PL mutations could cause a classic IP phenotype, further complicated by X-chromosome linkage and skewing of X inactivation.

Other aspects of IP may help explain disorders with similar problems, and the corresponding genes could implement their effects via NF-κB. For example, the most significant medical problem in IP is blindness due to retinal detachment, which is likely to result from abnormal vascularization. Various retinal diseases are attributed to neovascularization, including diabetic retinopathy, vein occlusion, and retinopathy of prematurity (ROP). Inhibition of VEGFs has been shown to reduce this neovascularization [58]. Thus, genes that interact with VEGFs and impinge on the NF-κB pathway could cause disorders with IP-like retinal manifestations, such as ROP. Finally, there are several proteins that impinge on the NF-κB pathway, and their corresponding knockout models exhibit phenotypes that could relate to human diseases (Table 1). For instance, *p52* (*NF-κB2*) could be disrupted by chromosomal translocations, leading to leukemia and non-Hodgkin's lymphoma [59] (from OMIM 164012), possibly arising from misregulation of apoptosis. There is some evidence that *Rel A* (*p65*) may be involved in chronic intestinal inflammation and thus, may explain Crohn's disease-like symptoms [60]. The absence of *Traneε*, the gene that encodes OPGL, causes osteopetrosis, chondrodysplasia and anodontia in mice, and could potentially explain corresponding human disorders [61]. Finally, *Irak* (interleukin receptor-associated kinase) knockout mice have severe immune dysfunction [62,63], and an equivalent human condition could exist which resembles IP in some respect, since IRAK is X-linked and activates the NF-κB pathway via NEMO.

Conclusions

At this point, the key to understanding the roles of NF-κB in human diseases is to identify genes downstream of EDAR, TNFR, RANK and VEGFR3, and upstream of NF-κB. Defects in these genes are likely to cause various human disorders with similarities to those described in this review. Analysis of the downstream genes affected by NF-κB is also likely to provide significant insight into the function of this pathway and could illuminate connections to other human diseases. In this respect, PCR- or DNA-microarray-based comparisons between normal mice and NF-κB-defective mice may identify differentially expressed transcripts that are pertinent to NF-κB function in the various developmental programs highlighted in this review.

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The *Nemo (Ikk-γ)* knockout mouse showed an expected phenotype – the heterozygote females resemble the human incontinentia pigmenti (IP) females – and provide insight into the pathogenesis of the IP skin lesions and male lethality. The authors also present a hypothesis to explain the skin phenotype.

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The male lethality of the *Nemo* (*Ikk-γ*) knockout mouse was as expected, but the heterozygote females resemble the human incontinentia pigmenti (IP) females in phenotype and provide clues regarding the pathogenesis of IP. Although the DNA analysis data from human patients are unconvincing, there is a good demonstration of absent NF-κB activity in human patient fibroblasts, further supporting a role for *NEMO* (*IKK-γ*) in incontinentia pigmenti. The authors also propose a model to explain the causative mechanism for the skin lesions in *Nemo*$^{+/-}$ mice and human IP.

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We describe novel hypomorphic *NEMO* mutations in IP males – this work was also described at the American Society of Human Genetics meeting in Philadelphia in October 2000 – who presented atypical phenotypes, particularly immune dysfunction or hemorrhaging. Interestingly, affected female relatives of the male patients exhibited classic IP signs. This work shows that the exon 10 mutations are indeed hypomorphic by demonstrating random or slight skewing of X-inactivation and a reduction, and not elimination, of NF-κB activity. We offer some ideas to explain the variant phenotypes taking into account several recent publications described in this review.

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Describes rare male ectodermal dysplasia (ED) cases that did not have mutations in *ED1* although families transmitted disease in an X-linked fashion. These males had immune dysfunction (ID) and thus were tested for *NEMO* mutations because of the link between the NF-κB pathway and immune reactions. Identification of *NEMO* mutations in the last exon indicates that ED/ID (or variant forms of incontinentia pigment (IP)) is allelic with IP.

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Excellent description of molecular defects in males with ED/IP and immune dysfunction only or with a combination of ED/IP and osteopetrosis, lymphedema and immune dysfunction. The authors nicely describe that all of these males have mutations in exon 10 of *NEMO*, which does not eliminate NF-κB signaling completely. This allow complete phenotypic expression in these patients. The abnormal immune system problems arise from defective response to lipopolysaccharide and various cytokines.

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This is also a good description of molecular defects in males with ED/IP and immune dysfunction. The authors demonstrate that B cells from their patients fail to undergo immunoglobulin class-switch recombination, and that antigen-presenting cells are incapable of inducing the production of specific cytokines. These problems lead to the immune dysfunction. This report also shows that the mutations fail to induce the degradation of the inhibitory IκB complex, thereby affecting NF-κB function.

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